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Adipose Tissue Lipogenic Enzyme Activity, Serum IGF-I, and IGF-Binding Proteins in the Callipyge Lamb

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Abstract

The purpose of this study was to determine if reduced adipose tissue accretion in callipyge lambs during growth was related to activities of lipogenic enzymes and serum concentrations of insulin, insulin-like growth factor-I (IGF-I), and IGF-binding proteins. Normal lambs were homozygous normal (clpg/clpg), and callipyge lambs were heterozygotes (CLPG/clpg). Lambs were slaughtered at 25, 41, 57, or 73 kg (target live weight groups), with five normal and five callipyge lambs in each weight group. Subcutaneous, intermuscular, and perirenal adipose tissue samples were dissected as soon as possible after slaughter from the 41, 57, and 73 kg groups. Tissue homogenates were prepared for assay of fatty acid synthase, acyl-CoA synthetase, glycerophosphate acyltransferase, and lipoprotein lipase activities. Most numeric values for enzyme activities were higher for the normal lambs in each adipose tissue depot. Callipyge lambs had lower ($P = 0.05$) glycerophosphate acyltransferase activity in subcutaneous adipose tissue at 41 kg. In intermuscular adipose tissue, each enzyme activity was lower ($P \leq 0.05$) at 41 kg for callipyge lambs. In perirenal adipose tissue, fatty acid synthase and glycerophosphate acyltransferase activities were lower ($P = 0.02$) for callipyge lambs at 41 kg, and acyl-CoA synthetase was lower ($P = 0.02$) for callipyge lambs at 73 kg.. Serum concentrations of insulin were not affected by genotype ($P > 0.20$). Serum insulin in non-fasted callipyge lambs was not affected by body weight, but increased with weight in non-fasted normal lambs ($P = 0.03$). Two-day fasted lambs had decreased serum insulin in both genotypes, which increased ($P = 0.03$) similarly with body weight for both genotypes. Serum IGF-I was greater ($P = 0.09$) in normal lambs at 73 kg, whereas IGF-I in 2-d fasted callipyge lambs was greater ($P = 0.03$) than normal lambs. No genotype effects were observed for the relative proportions of the IGF-binding proteins. We conclude that callipyge lambs had lower lipogenic enzyme activities in adipose tissue than normal lambs, but these changes were not related to serum concentrations of insulin or IGF-I.

Key words: lambs; callipyge; lipogenesis; insulin

Introduction

The callipyge phenotype is an inheritable neomutation, and the result of a single dominant autosomal gene (Cockett et al., 1994; Cockett et al., 1996). Although growth rates of callipyge lambs were similar to normal lambs (Jackson et al., 1997a; Freking et al., 1998), callipyge lambs were more muscular, especially in the hind quarters, and lower in fat depth than normal lambs (Jackson et al., 1997b; 1997c; Freking et al., 1998). Biochemical characterization of the callipyge lamb has been limited to muscle, with the calpain/calpastatin system the most thoroughly studied (Koohmaraie et al., 1995). Collagen and collagen crosslinking also have been reported (Field et al., 1996). To date no data on adipose tissue biochemistry have been reported for the callipyge lamb. The reduced fat deposition previously reported for callipyge lambs suggests that lipid accretion was down regulated; however, the mechanism remains unknown. We hypothesize that adipose tissue lipogenic enzyme activity is lower in callipyge than in normal lambs. The purpose of the present study was to determine if lipogenic enzyme activity in ovine adipose tissue is influenced by the callipyge genotype, and to determine if changes are related to insulin, insulin-like growth factor-I (IGF-I), and IGF-binding proteins in serum.

Materials and Methods

Animals.

A serial slaughter of 40 wether lambs weighing from 18.2 to 74.3 kg was conducted. Lambs were the offspring from heterozygous callipygous Dorset/Columbia rams (CLPG/clpg) mated to normal Columbia ewes (clpg/clpg). Because the callipyge phenotype cannot be distinguished accurately before 10 wk of age, lambs under 25 kg were genotyped with microsatellites flanking the CLPG locus (Cockett et al., 1994; Cockett et al., 1996). Lambs over 25 kg were phenotyped for the heavy muscling characteristic of the callipyge expression or for normal muscling.

Lambs were reared with their dams under

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confinement until reaching slaughter weight or weaned at about 90 d postpartum. Ewes and lambs were fed chopped alfalfa hay ad libitum, and the ewes were supplemented daily with 0.5 kg of whole barley. Lambs had ad libitum access to a commercial lamb creep feed until about 90 d of age. After weaning, lambs were adjusted to a diet of 60% whole barley and 40% pelleted alfalfa offered for ad libitum consumption. Lambs were randomly preassigned to a slaughter weight group. Appropriate guidelines for humane lamb slaughter were implemented.

Tissue sampling.
Adipose tissue was only sampled from lambs slaughtered at target live weights of 41, 57, and 73 kg because the subcutaneous depot of 25-kg lambs could not provide a sufficient tissue sample. Subcutaneous, intermuscular, and perirenal adipose tissue samples were obtained immediately after stunning, exsanguinating, and removing the pelt. Subcutaneous adipose tissue was dissected adjacent the 12th rib, and intermuscular adipose tissue was sampled from around the prescapular lymph node (beneath the trapezius muscle). Tissue samples were wrapped in aluminum foil, and placed in liquid nitrogen. Samples were stored at -70° C until analyzed (within 12 wk).

Ten d before slaughter, jugular venous blood samples were obtained from each lamb of the 25, 41, 57, and 73-kg target live weight groups. Lambs were then fasted for 48 h, after which a second jugular venous blood sample was obtained. Lambs were then provided ad libitum access to their diet until slaughter. Serum was harvested for analysis of insulin, IGF-I, and IGF-binding proteins.

Adipose tissue and serum assays.
Activities of fatty acid synthase, acyl-CoA synthetase, glycerophosphate acyltransferase, and lipoprotein lipase were determined in tissue homogenates. Fatty acid synthase activity was determined by quantifying the rate of oxidation of NADPH (Vernon, 1976; Vernon and Taylor, 1986). Acyl-CoA synthetase activity was quantified by determining the rate of palmitate conversion to palmitoyl-CoA (Shimamura et al., 1992). Glycerophosphate acyltransferase activity was determined by measuring the rate of incorporation of palmitoyl-CoA into total glycerolipids (Rule et al., 1988a). Total lipoprotein lipase activity was determined according to Andersen et al. (1996). All data were expressed per mg of homogenate protein, as assayed using the biuret procedure. Tissue freezing in liquid N did not affect glycerolipid biosynthesis activity in swine adipose tissue (Rule et al., 1988b). By using protease inhibitor for preparation of lipoprotein lipase activity measurements, activity in frozen preparations was not inhibited (Rule et al., 1996). Fatty acid synthase activity was not lost by tissue freezing in preliminary experiments.

Serum concentrations of IGF-I were determined in duplicate as described previously (Echternkamp et al., 1990; Funston et al., 1995a; Clapper et al., 1998). Relative amounts of IGF-binding proteins in serum were analyzed by one-dimensional SDS-PAGE (Laemmli, 1970) and ligand blotting (Hossenloop et al., 1986; Howard and Ford, 1992) as described previously (Funston et al., 1995a; Clapper et al., 1998). Identities of IGF-binding proteins-2, -4, and –5 in serum were previously confirmed by immunoprecipitation (Clapper et al., 1998; Funston et al., 1996a,b). On the basis of the similarity in molecular masses of IGF-binding proteins identified in cattle (Funston et al., 1995; Roberts and Funston, 1993), the IGF-binding protein detected as a 40/44 kDa doublet was presumed to be IGF-binding protein-3. Band density of each IGF-binding protein was divided by the band density of the respective IGF-binding protein present in a standard ovine serum sample placed on each gel. These data, expressed as percentages of standard band densities for each IGF-binding protein, were compared to determine effects of treatment and genotype on each IGF-binding protein.

Statistical analysis.
Data for lipogenic enzymes were analyzed by three-way ANOVA to determine effects of genotype, body weight group, and tissue depot. Genotype effects across body weight groups and tissues depots were observed; therefore, genotype and body weight group effects within adipose depot were reported. Serum hormones and IGF-binding protein data were analyzed by repeated measures to ascertain the effects of feed restriction, and by two-way ANOVA to determine effects of genotype and body weight group. Statistical Analysis System (SAS, Version 5 Edition; 1985) was used for statistical computations. Effect of genotype was considered significant at $P < 0.10$; however, actual P values were noted for the readers' interpretation.

Results and Discussion

Live weights at slaughter were slightly lower than the target weights because of shrink caused by the overnight stand without feed before slaughter. Actual live weights (mean ± SEM) for the 25, 41, 57, and 73-kg groups, respectively, were as follows: 22.1 ± 0.6 , 37.7 ± 0.8, 52.5 ± 0.9, and 70.4 ± 2.3 for the callipyge lambs, and 18.7 ± 0.5, 35.6 ±0 .9, 53.8 ± 1.2, and 67.9 ± 1.1 for the normal lambs. The mean age within target weight groups were similar for normal and callipyge lambs (25-kg: 100 vs. 103 d; 41-kg: 148 vs. 147 d; 57-kg: 163 vs. 160 d; 73-kg: 246 vs. 244 d, respectively). Expression of the callipyge gene did not affect growth rate when normal and callipyge lambs were fed similar diets (Snowder et al., 1994; Jackson et al., 1997b).

Lipogenic enzyme activities.
Across body weight group and adipose tissue depot, genotype effects were observed for fatty acid synthase ($P = 0.02$), acyl-CoA synthetase ($P = 0.02$), glycerophosphate acyltransferase ($P = 0.009$), and lipoprotein lipase ($P = 0.10$), with callipyge lambs having the lower activity for each enzyme. Across genotype and adipose tissue depot, body weight group effects ($P \leq 0.03$) were observed for each enzyme. Fatty acid synthase and acyl-CoA synthetase activities were lowest at 41 kg, intermediate at 57 kg, and highest at 73 kg. Glycerophosphate acyltransferase and lipoprotein lipase activities were highest at 57 kg, and lowest and 41 and 73 kg; these enzyme activities were similar at the latter two body weights. Across genotype and body weight group, adipose tissue depot effects were observed for each enzyme ($P < 0.001$) except for glycerophosphate acyltransferase ($P = 0.23$). Fatty acid synthase and acyl-CoA synthetase activities were highest in perirenal adipose tissue, but were similar for subcutaneous and intermuscular adipose tissue, and lipoprotein lipase activity was lowest in subcutaneous adipose tissue, but similar for perirenal and intermuscular adipose tissues. No genotype x adipose tissue depot interactions were observed (P =

0.13 to 0.89). No genotype x body weight group x adipose tissue depot interactions were observed (P = 0.08 to 0.64). A genotype x body weight group interaction was observed for glycerophosphate acyltransferase (P < 0.001), but not for the other enzyme activities (P = 0.21 to 0. 90).

Lipogenic enzyme activities in callipyge and normal lambs for the three adipose tissue depots are presented in Tables 1-3. Except for acyl-CoA synthetase at 41 kg and glycerophosphate acyltransferase at 57 kg, numeric values for subcutaneous adipose tissue lipogenic enzyme activities (Table 1) were higher in normal lambs than in callipyge lambs. Fatty acid synthase activity in subcutaneous adipose tissue tended to be greater in normal lambs with the greatest difference expressed at 73 kg (P = 0.12). Glycerophosphate acyltransferase activity in subcutaneous adipose tissue was greater for normal lambs at 41 kg (P = 0.05), but tended to be greater for normal lambs at 73 kg (P = 0.11). In contrast, glycerophosphate acyltransferase activity in subcutaneous adipose tissue of 57 kg callipyge lambs tended to be greater (P = 0.08) than that in the normal lambs. Lipoprotein lipase activity in subcutaneous adipose tissue tended to be greater in normal lambs (P values ranged from 0.12 to 0.54). Overall, lipogenic enzyme activities of fatty acid synthase, acyl-CoA synthetase, and lipoprotein lipase in subcutaneous adipose tissue increased with increasing weight with higher values generally observed in normal lambs compared to callipyge lambs. Glycerophosphate acyltransferase activity in callipyge lambs was similar at 57 and 73 kg (P > 0.05), but in normal lambs, this enzyme activity increased by nearly 60% (P < 0.05) between 57 and 73 kg.

Similar to subcutaneous adipose tissue, numeric values for enzyme activities in intermuscular adipose tissue were generally greater in normal than in callipyge lambs, except for fatty acid synthase at 73 kg, which tended to be greater (P = 0.09) in callipyge than in normal lambs (Table 2). For each enzyme in intermuscular adipose tissue, normal lambs had greater activity (P = 0.05 to 0.001) than callipyge lambs at 41 kg. Except for fatty acid synthase activity at 73 kg, no genotypic differences (P ≥ 0.24) in enzyme activities were observed at 57 and 73 kg.

Table 1. Lipogenic enzyme activity[a] in subcutaneous adipose tissue of callipyge and normal lambs from 41 to 73 kg

Enzyme	Weight group, kg	Genotype: Callipyge	Normal	SE[b]	*P*-value[b]
Fatty acid synthase	41	17.8[d]	27.7[d]	8.0	0.41
	57	78.0[c]	87.6[c]	15.0	0.66
	73	81.3[c]	130.2[c]	19.9	0.12
Acyl-CoA synthetase	41	46.5[d]	41.2[e]	3.6	0.34
	57	59.6[d]	67.4[d]	5.4	0.34
	73	103.5[c]	115.0[c]	11.0	0.50
Glycerophosphate acyltransferase	41	33.3[d]	47.4[d]	4.3	0.05
	57	64.2[c]	47.2[d]	5.8	0.08
	73	55.9[c]	75.4[c]	7.5	0.11
Lipoprotein lipase	41	31.0[d]	39.1[d]	4.0	0.16
	57	56.1[c]	64.0[c]	9.0	0.54
	73	61.0[c]	81.1[c]	8.1	0.12

[a]Units of enzyme activity were as follows: Fatty acid synthase, nMol NADPH oxidized • 15 min^{-1} • mg of homogenate $protein^{-1}$; acyl-CoA synthetase, nMol palmitate converted to palmitoyl-CoA • 10 min^{-1} • mg of homogenate $protein^{-1}$; glycerophosphate acyltransferase, nMol glycerol-3 phosphate converted to glycerolipid • 10 min^{-1} • mg homogenate $protein^{-1}$; lipoprotein lipase, nEquivalents of fatty acid released • 10 min^{-1} • mg of homogenate $protein^{-1}$.
[b]Standard error (SE) and probability (P) values were determined by ANOVA for comparison of genotype within a body weight group. N=5 per genotype within each weight group.
[c,d,e] Within a column, for each enzyme, genotypic means without a common superscript letter differ within weight groups (P < 0.05).

Table 2. Lipogenic enzyme activity[a] in intermuscular adipose tissue of callipyge and normal lambs from 41 to 73 kg

Enzyme	Weight group, kg	Genotype: Callipyge	Normal	SE[b]	*P*-value[b]
Fatty acid synthase	41	33.4[d]	64.7[e]	2.7	0.001
	57	93.9[c]	107.2[c]	8.1	0.28
	73	108.4[c]	86.4[d]	8.0	0 .09
Acyl-CoA synthetase	41	27.4[e]	41.4[d]	4.4	0.05
	57	58.2[d]	61.3[d]	5.7	0 .71
	73	83.7[c]	117.4[c]	18.3	0.24
Glycerophosphate acyltransferase	41	36.0[d]	59.4[c]	2.5	0 .001
	57	60.0[c]	63.6[c]	5.8	0 .66
	73	43.5[d]	40.9[d]	5.1	0 .73
Lipoprotein lipase	41	53.0[d]	89.1[c]	7.9	0.02
	57	89.2[c]	79.9[cd]	9.9	0 .54
	73	76.1[cd]	61.9[d]	9.1	0 .33

[a]Units of enzyme activity were as follows: Fatty acid synthase, nMol NADPH oxidized • 15 min^{-1} • mg of homogenate $protein^{-1}$; acyl-CoA synthetase, nMol palmitate converted to palmitoyl-CoA • 10 min^{-1} • mg of homogenate $protein^{-1}$; glycerophosphate acyltransferase, nMol glycerol-3 phosphate converted to glycerolipid • 10 min^{-1} • mg homogenate $protein^{-1}$; lipoprotein lipase, nEquivalents of fatty acid released • 10 min^{-1} • mg of homogenate $protein^{-1}$.
[b]Standard error (SE) and probability (P) values were determined by ANOVA for comparison of genotype within a body weight group. N=5 per genotype within each weight group.
[c,d,e] Within a column, for each enzyme, genotypic means without a common superscript letter differ within weight groups (P < 0.05).

Table 3. Lipogenic enzyme activity[a] in perirenal adipose tissue of callipyge and normal lambs from 41 to 73 kg

Enzyme	Weight group, kg	Genotype		SE[b]	*P*-value[b]
		Callipyge	Normal		
Fatty acid synthase	41	56.1[e]	84.1[d]	6.8	0 .02
	57	124.5[d]	147.8[c]	9.7	0 .13
	73	157.9[c]	180.7[c]	16.1	0 .34
Acyl-CoA synthetase	41	50.5[e]	64.2[e]	6.8	0 .19
	57	79.4[d]	118.2[d]	12.4	0.06
	73	119.2[c]	152.3[c]	8.2	0.02
Glycerophosphate acyltransferase	41	38.1[d]	62.8[cd]	6.0	0 .02
	57	79.7[c]	67.9[c]	6.0	0.20
	73	36.2[d]	42.4[d]	6.5	0.52
Lipoprotein lipase	41	79.0	93.9[cd]	13.0	0 .44
	57	87.0	102.2[c]	10.1	0 .33
	73	75.9	66.1[d]	8.9	0.47

[a]Units of enzyme activity were as follows: Fatty acid synthase, nMol NADPH oxidized • 15 min^{-1} • mg of homogenate $protein^{-1}$; acyl-CoA synthetase, nMol palmitate converted to palmitoyl-CoA • 10 min^{-1} • mg of homogenate $protein^{-1}$; glycerophosphate acyltransferase, nMol $glycerol^{-3}$ phosphate converted to glycerolipid • 10 min^{-1} • mg homogenate $protein^{-1}$; lipoprotein lipase, nEquivalents of fatty acid released • min^{-1} • 10 mg of homogenate $protein^{-1}$.
[b]Standard error (SE) and probability (P) values were determined by ANOVA for comparison of genotype within a body weight group. N=5 per genotype within each weight group.
[c,d,e] Within a column, for each enzyme, genotypic means without a common superscript letter differ within weight (P < 0.05).

In perirenal adipose tissue, fatty acid synthase activity was greater (P = 0.02) in normal lambs at 41 kg, and tended (P = 0.13) to be greater in normal lambs at 57 kg (Table 3). Acyl-CoA synthetase activity in perirenal adipose tissue was greater in normal lambs at 57 (P = 0.06) and at 73 kg (P = 0.02). Glycerophosphate acyltransferase activity was greater (P = 0.02) in normal lambs at 41 kg, but did not differ at 57 and 73 kg. Lipoprotein lipase activity in perirenal adipose tissue did not differ between normal and callipyge lambs. Across weight groups, activities of fatty acid synthase and acyl-CoA synthetase increased (P < 0.05) with increasing weight in perirenal adipose tissue. Across adipose tissue depots, lipogenic enzyme activity was greater in perirenal adipose tissue compared to subcutaneous (Table 1) and intermuscular adipose tissues (Table 2).

Expressing lipogenic enzyme activity on a protein basis accounts for potential differences in adipose tissue cellularity because as adipocytes decrease in volume, the protein concentration should increase, as well as the number of adipocytes per gram of tissue. Adipose tissue homogenate protein concentration across weight group was not affected by genotype for either subcutaneous (P = 0.85) or perirenal (P = 0.15) adipose tissues. For perirenal adipose tissue across genotypes, protein concentration was greatest (P < 0.001) for the 41 kg lambs (7.78 mg/ g tissue), whereas, protein concentration for the 57 kg lambs (4.10 mg/g tissue) tended (P = 0.16) to be greater than for the 73 kg lambs (2.80 mg/ g tissue). In subcutaneous adipose tissue across genotype, protein concentration for the 41 kg lambs (9.88 mg/ g tissue) was greatest (P = 0.003), intermediate for the 57 kg lambs (5.98 mg/ g tissue), and lowest (P = 0.05) for the 73 kg lambs (3.58 mg/ g tissue). In the intermuscular adipose tissue depot, an interaction (P = 0.008) between genotype and weight group was observed. The 41 kg lambs had greater (P < 0.001) protein concentration (8.20 mg/ g tissue) than either the 57 kg lambs (4.32 mg/ g tissue) or the 73 kg lambs (3.55 mg/ g tissue), both of which were similar (P = 0.42). The 41 kg callipyge lambs had over twofold the protein concentration (11.08 mg/ g tissue) of the other genotype and weight groups, all of which had similar (P ≥ 0.42) protein concentration in the intermuscular depot (3.50 to 5.32 mg/ g tissue). For each genotype and tissue depot, the effect of weight group suggests that as animal weight increased protein concentration decreased, which also would be expected for adipocyte number per g of tissue. Expressing data on a tissue weight basis (data not shown) altered few of the genotype comparisons, with the major exception being the 41 kg callipyge lambs in the intermuscular depot where on a tissue weight basis genotype effects were diminished. Thus, expressing the data on a protein basis proved to be an acceptable comparison of normal and callipyge lamb lipogenic enzyme activity.

Our data indicate a general depression in lipogenic enzyme activity across adipose tissue depots and at various body weights of callipyge compared with normal lambs. Callipyge lambs used in the present study were leaner over the growth curve than the normal lambs (Snowder et al., 1998). Thus, lower fat deposition during growth in callipyge lambs can be attributed, in part, to lower lipogenic enzyme activities than in normal lambs. However, the mechanism by which lipogenic enzyme activities were decreased cannot be discerned from the present study. Genetic predisposition to lower rates of lipid accretion may partially characterize callipyge lambs because models of genetic obesity, such as porcine obesity, have greater lipogenesis in adipose tissue at various times during postnatal growth (Mersmann, 1986). Moreover, adipose tissue of Meishan pigs (an obese model) had greater activities of lipogenic enzymes, especially during periods of greatest fat deposition (Mourot et al., 1996). In Zucker obese rats, lipogenic enzyme activity in adipose tissue was greater than in lean rats even during periods of feed restriction (Cleary et al., 1987). Based on these examples, lower lipogenic enzyme activities in callipyge adipose tissue may have been the result of genetic down-regulation of lipogenesis compared with their normal counterparts.

Alternatively, the depressed enzyme activities observed in callipyge adipose tissue may have occurred through increased cellular

utilization of substrate supply by muscle, leaving adipose tissue in a state of reduced substrate availability. Pothoven and Beitz (1975) clearly demonstrated a reduction in lipogenesis and lipogenic enzyme activity in adipose tissue of fasted cattle. In response to fasting, endocrine and metabolite changes occur rapidly in cattle (Rule et al., 1985), as well as in sheep as illustrated in the present study with insulin (Table 4). Feed restriction, as opposed to fasting, also reduces activities of lipoprotein lipase and glycerophosphate acyltransferase in adipose tissue of growing lambs (Andersen et al., 1996). In the present study, the most pronounced differences in enzyme activities occurred at the lighter weights, suggesting that at 41 kg, the muscular hypertrophy occurring in the callipyge lambs may have resulted in the greatest competition with adipose tissue for lipogenic substrates.

Serum concentrations of insulin, IGF-I, and IGF-binding proteins.

No genotypic effect on non-fasted or fasted serum concentrations of insulin (Table 4) were observed at any of the body weights studied (P > 0.20). For both callipyge and normal lambs, fasted insulin values were lower than the non-fasted values (P < 0.05). For the non-fasted callipyge lambs, serum insulin concentrations were not affected by body weight group (P = 0.19); however, fasted insulin concentrations were greater (P < 0.01) for the 57 and 73 kg callipyge lambs than for the 25 and 41 kg callipyge lambs. In normal lambs, serum insulin concentrations increased with body weight for both non-fasted values (P = 0.03) and fasted values (P = 0.01). Serum insulin concentrations in non-fasted callipyge and normal lambs also were not different in a previous study (Whisnant et al., 1998).

Genotype effects on IGF-I concentrations were observed only for the 73-kg lambs (Table 4). For non-fasted lambs, callipyge had lower IGF-I values (P = 0.09) than normal lambs. After fasting, however, IGF-I concentrations in callipyge lambs were greater (P = 0.03) than in normal lambs. For both genotypes, fed IGF-I concentrations increased (P < 0.01) with development from 25 to 41 kg and did not change thereafter for callipyge, but continued to increase (P < 0.01) for normal lambs. For fasted lambs, IGF-I concentrations tended to continually increase for the callipyge lambs, but for the normal lambs, fasted IGF-I reached a plateau by 41 kg of live weight. Similar observations were reported for callipyge compared with normal lambs in the fed state (Hossner et al., 1995; Whisnant et al., 1998).

Table 4. Insulin and insulin-like growth factor-I (IGF-I) concentrations in non-fasted and fasted callipyge and normal lambs from 25 to 73 kg

Weight group, kg	Genotype[a]			
	Callipyge		Normal	
	Non-fasted	Fasted	Non-fasted	Fasted
Insulin, μIU/mL				
25	10.9	$5.6^{c,x}$	10.9^{d}	$6.9^{c,x}$
41	13.8	$7.8^{c,x}$	$12.0^{c,d}$	$6.8^{c,x}$
57	13.6	11.2^{b}	$15.0^{b,c}$	$10.3^{a,y}$
73	14.1	$11.7^{b,y}$	16.7^{b}	12.1^{a}
SE	1.1	0 .8	1.3	1.1
IGF-I, ng/mL				
25	41.8^{d}	25.8^{d}	38.3^{d}	$20.2^{c,y}$
41	125.3^{b}	$81.6^{c,y}$	$119.7^{b,c}$	$69.5^{b,y}$
57	$92.2^{b,c}$	$104.4^{b,c}$	84.9^{c}	86.0^{b}
73	$75.2^{c,d}$	117.5^{b}	132.6^{b}	$76.3^{b,y}$
SE	13.4	8.4	13.8	10.3

[a]No effect of genotype (P > 0.2) was observed for non-fasted or fasted serum insulin concentrations. For IGF-I, genotype effects were observed only for the 73 kg lambs, in which IGF-I was greater for normal lambs (P < 0.09), but fasted values were lower for normal lambs (P = 0.03) than for the callipyge lambs.
[b,c,d] Within a column, means without a common superscript letter differ within weight groups (P < 0.03 for insulin, and P < 0.01 for IGF-I).
[x]Within a column depicting common genotypic means (either callipyge or normal), non-fasted values were different from fasted values (P < 0.01).
[y]Within a column depicting common genotypic means (either callipyge or normal), non-fasted values were different from fasted values (P < 0.05).

Six IGF-binding proteins were detected in serum. On the basis of immunoprecipitation in previous studies (Roberts and Funston, 1993; Funston et al., 1995a,b; Funston et al., 1996; Clapper et al., 1998; Snyder et al., 1999), these proteins were identified as a 29 kDa IGF-binding protein (presumably IGF-binding protein-1; Gallaher et al., 1992), IGF-binding protein-2 (34 kDa), IGF-binding protein-3 (40 and 44 kDa), and IGF-binding protein-4 (24 and 28 kDa). Standardized band densities for each IGF-binding protein were expressed as a percentage of the respective IGF-binding protein in a standard ovine serum sample in Table 5. No genotypic effect was observed for IGF-binding proteins for either non-fasted or fasted lambs (P > 0.20). This result contrasts that reported by Hossner et al. (1995) wherein feed restriction reduced IGF-I binding to IGF-binding protein-4 by 55% in normal lambs, but not in callipyge lambs.

Fasting resulted in reductions (P < 0.01) in the proportions of IGF-binding proteins-4, and the 29 kDa binding protein; whereas, fasting resulted in increased proportions of the 40 and 44 kDa IGF-binding protein-3. No effect of fasting was observed for IGF-binding protein-2. In serum of non-fasted lambs, proportions of the 24 and 28 kDa IGF-binding proteins increased (P < 0.05)

Table 5. Standardized IGF-binding protein percentages in non-fasted lambs and after 48 hours of fasting, from 25 to 73 kg

Condition	Weight group, kg	IGF Binding Proteins[a] BP-4 24 kDa	BP-4 28 kDa	BP-3 40 kDa	BP-3 44 kDa	BP-2	BP 29 kDa
Non-fasted	25	20.7[d]	26.9[d]	35.0[c]	3.8	4.4	9.3
	41	27.8[c]	38.0[c]	17.4[d]	3.3	5.2	8.4
	57	27.4[c]	33.0[cd]	20.0[d]	4.9	6.2	8.5
	73	29.1[c]	34.8[c]	21.3[d]	3.1	4.4	7.3
	SE	2.4	2.6	4.6	0 .7	0 .6	1.0
Fasted	25	8.4[e]	9.3[d]	58.0[c]	13.0[c]	5.6	5.6
	41	16.7[d]	20.7[c]	43.1[d]	7.9[d]	5.4	6.2
	57	19.7[de]	23.4[c]	36.8[d]	7.2[d]	5.7	7.2
	73	23.9[c]	27.1[c]	31.6[d]	6.3[d]	4.9	6.2
	SE	2.2	2.7	4.5	1.1	0.4	0.5

[a]Pixel density for each IGF-binding protein/pixel density for the respective IGF-binding protein in a standard ovine sereum sample x 100.
[b]Fed was different from fasted ($P < 0.01$) for each BP except for BP-2 ($P = 0.31$).
[c,d,e]Within a condition, columns with weight group means with different superscript letters differ ($P < 0.05$ for non-fasted lambs and $P < 0.01$ for fasted lambs).

from 25 to 41 kg of live weight, whereas the proportion of the 40 kDa binding protein-3 decreased ($P < 0.05$). Similar types of responses ($P < 0.01$) to growth occurred for the IGF-binding proteins after fasting, except that the proportion of the 44 kDa binding protein-3 decreased from 25 to 41 kg of live weight ($P < 0.01$).

The major changes in proportions of the IGF-binding proteins occurred from 25 to 41 kg of body weight. IGF-I concentrations also changed to the greatest extent between 25 and 41 kg of body weight. Because components of the IGF-I system may change independently of changes in the circulation, it remains possible that IGF-I and IGF-binding proteins in discrete tissues could differ among callipyge and normal lambs and thereby influence growth and fat accretion in a paracrine or autocrine fashion (McGuire et al.,1992; Jones and Clemmons, 1995; Snyder et al., 1999).

The relationships between insulin, IGF-I, and IGF-binding proteins with lipogenic enzymes were not readily apparent in the present study. Little, if any, effect of genotype occurred for the serum hormones measured, whereas callipyge adipose tissue had lower lipogenic enzyme activities. .

In conclusion, lipogenic enzyme activities in adipose tissue of callipyge lambs were generally lower than normal lambs. This observation may partially explain the reduced fat deposition common to callipyge lambs during growth, but the mechanism of the lower enzyme activities remains unclear. Little effect of genotype on serum insulin, IGF-I, and IGF-binding proteins was observed; however, responsiveness of adipose tissue or muscle to these hormones is not known. Further research on growth factor effectiveness and IGF-binding proteins in muscle is needed to determine how these elements affect the growth differential between callipyge and normal lambs.

Implications

The callipyge lamb offers consumers a leaner, more muscular alternative to common lamb. Moreover, the callipyge lamb may also represent a model for a genetically lean animal. The lower lipogenic enzyme activities partially explain the lean qualities of the callipyge lamb; however, further research is necessary to elucidate the mechanism for the enzymatic changes, as well as the role growth factors play in expression of the callipyge phenotype.

Literature Cited

Andersen, M. K., J. W. Bailey, C. Wilken, and D. C. Rule. 1996. Lipoprotein lipase and glycerophosphate acyltransferase in ovine tissues are influenced by growth and energy intake regimen. J. Nutr. Biochem. 7:610-616.

Clapper, J. A., J. L. Snyder, A. J. Roberts,

D. L. Hamernik, and G. E. Moss. 1998. Estradiol increases relative amounts of insulin-like growth factor binding protein (IGFBP)-3 in serum and expression of IGFBP-2 in anterior pituitaries of ewes. Biol. Repro. 59:124-130.

Cleary, M. P., S. Muller, and S. Lanza-Jacoby. 1987. Effects of long-term moderate food restriction on growth, serum factors, lipogenic enzymes and adipocyte glucose metabolism in lean and obese Zucker rats. J. Nutr. 117:355-360.

Cockett, N. E., S. P. Jackson, T. L. Shay, D. Nielsen, S. S. Moore, M. R. Steele, W. Barendse, R. D. Green, and M. Georges. 1994. Chromosomal localization of the callipyge gene in sheep (Ovis aries) using bovine DNA markers. Proc. Natl. Acad. Sci. 91:3019-3023.

Cockett, N. E., S. P. Jackson, T.L. Shay, F. Farnir, S. Berghmans, G. D. Snowder, D. Nielsen, and M. Georges. 1996. Polar overdominance at the ovine callipyge locus. Science 273:236-238.

De la Hoz, L. and R. G. Vernon. 1993. Endocrine control sheep adipose tissue fatty acid synthesis: depot specific differences in response to lactation. Horm. Metab. Res. 25:214-218.

Echternkamp, S. E., L. J. Spicer, K. e. Gregory, S. F. Canning, and J. M. Hammond. 1990. Concentrations of insulin-like growth factor-I in blood and ovarian follicular fluid of cattle selected for twins. Biol. Reprod. 43:8-14.

Field, R. A., R. J. McCormick, D. R. Brown, F. C. Hinds, and G. D. Snowder. 1996. Collagen crosslinks in longissimus muscle from lambs expressing the callipyge gene. J. Anim. Sci. 74:2943-2947.

Freking, B. A., J. W. Keele, M. K. Nielsen, and K. A. Leymaster. 1998. Evaluation of the ovine callipyge locus: II. Genotypic effects on growth, slaughter, and carcass traits. J. Anim. Sci. 76:2549-2559.

Funston, R. N., G. E. Moss, and A. J. Roberts. 1995a. Insulin-like growth factor-I (IGF-I) and IGF-binding proteins in bovine sera and pituitaries at different stages of the estrous cycle. Endocrinology 136:62-68.

Funston, R. N., A. J. Roberts, D. L. Hixon, D. M. Hallford, D. S. Sanson, and G. E. Moss. 1995b. Effect of acute glucose antagonism on hypophyseal hormones and concentrations of insulin-like growth factor (IGF)-I and IGF-binding proteins in serum, anterior pituitary, and hypothalamus of ewes. Biol. Reprod. 52:1179-1186.

Funston, R. N., G. E. Siedel, J. Klindt, and A. J. Roberts. 1996. Insulin-like growth factor-I and insulin-like growth factor-binding proteins in bovine serum and follicular fluid before and after preovulatory surge of luteinizing hormone. Biol. Reprod. 55:1390-1396.

Gallaher, B. W., B. H. Breier, M. H. Oliver, and J. E. Harding. 1992. Ontogenic differences in the nutritional regulation of circulating IGF-binding proteins in sheep plasma. Acta Endocrinol. 126:49-54.

Hossenloop, P., D. Seurin, B. Segovia-Quinson, S. Hardouin, and M. Binoux. 1986. Analysis of serum insulin-like growth factor binding proteins using Western blotting: use of the method for titration of the binding proteins and competitive binding studies. Anal. Biochem. 154:138-143.

Hossner, K. L., R. S. Yemm, G. Bernal-Santos, G. D. Snowder, and D. E. Johnson. 1995. Alteration of insulin and the insulin-like growth factor I system in sheep. J. Anim. Sci. 73(Suppl.1):138.

Howard, H. J. and J. J. Ford. 1992. Relationships among steroids, inhibin, insulin-like growth factor-I (IGF-I), and IGF-binding proteins during follicular development in weaned sows. Biol. Reprod. 47:193-201.

Jackson, S. P., R. D. Green, and M. F. Miller. 1997a. Phenotypic characterization of Rambouillet sheep expressing the callipyge gene: I. Inheritance of the condition and production characteristics. J. Anim. Sci. 75:14-18.

Jackson, S. P., M. F. Miller, and R. D. Green. 1997b. Phenotypic characterization of Rambouillet sheep expressing the callipyge gene: II. Carcass characteristics and retail yield. J. Anim. Sci. 75:125-132.

Jackson, S. P., M. F. Miller, and R. D. Green. 1997c. Phenotypic characterization of Rambouillet sheep expressing the callipyge gene: III. Muscle weights and muscle weight distribution. J. Anim. Sci. 75:133-138.

Jones, J. I. and D. R. Clemmons. 1995. Insulin-like growth factors and their binding proteins: biological actions. Endocr. Rev. 16:3-34.

Koohmaraie, M., S. D. Shackelford, T. L. Wheeler, S. M. Lonergan, and M. E. Doumit. 1995. A muscle hypertrophy condition in lamb (callipyge): Characterization of effects on muscle growth and meat quality traits. J. Anim. Sci. 73:3596-3607.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

McGuire, M. A., J. L. Vicini, D. E. Bauman, and J. J. Veenhuizen. 1992. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. J. Anim. Sci. 70:2901-2910.

McNamara, J. P. 1997. Adipose tissue metabolism during lactation: where do we go from here? Proc. Nutr. Soc. 56:149-167.

Mersmann, H. J. 1986. Postnatal expression of adipose tissue metabolic activity associated with a porcine genetic obesity. J. Anim. Sci. 63:741-746.

Mourot, J., M. Kouba, and M. Bonneau. 1996. Comparative study of in vitro lipogenesis in various adipose tissues in the growing Meishan pig: comparison with the Large White pig (Sus domesticus). Comp. Biochem. Physiol. 115B:383-388.

Pothoven, M. A. and D. C. Beitz. 1975. Changes in fatty acid synthesis and lipogenic enzymes in adipose tissue from fasted and fasted-refed steers. J. Nutr. 105:1055-1061.

Roberts, A. J. and R. N. Funston. 1993. Regulation of pituitary insulin-like growth factor binding proteins (IGF-BPs) by gonadotropin releasing hormone (LHRH). Biol. Reprod. 48 (Suppl. 1):186.

Rule, D. C., M. K. Andersen, L. A. Swain, S. J. Ficek, and d. PO. Thomas. 1996. Frozen storage of ovine and rat tissues adversely affects lipoprotein lipase activity. J. Nutr. Biochem. 7:577-581.

Rule, D. C., D. C. Beitz, G. de Boer, R. R. Lyle, A. H. Trenkle, and J. W. Young. 1985. Changes in hormone and metabolite concentrations in plasma of

steers during a prolonged fast. J. Anim. Sci. 61:868-875.
Rule, D. C., S. B. Smith, and H. J. Mersmann. 1988a. Glycerolipid biosynthesis in porcine adipose tissue in vitro. 1. Assay conditions for homogenates. J. Anim. Sci. 66: 1656-1664.
Rule, D. C., S. B. Smith, and H. J. Mersmann. 1988b. Glycerolipid biosynthesis in swine adipose tissue in vitro. II. Synthesis by various types of cellular preparations. J. Anim. Sci. 66:1665-1675.
Shimamura, I., K. Tokunaga, S. Jiao, T. Funahashi, Y. Keno, T. Kobatake, K. Kotani, H. Suzuki, T. Yamamoto, S. Tarui, and Y. Matsuzawa. 1992. Marked enhancement of acyl-CoA synthetase activity in mRNA, paralleled to lipoprotein lipase mRNA, in adipose tissues of Zucker obese rats (fa/fa). Biochim. Biophys. Acta 1124:112-118.
Snowder, G. D., J. R. Busboom, N. E. Cockett, F. Hendrix, and V. T. Mendenhall. 1994. Effect of the callipyge gene on lamb growth and carcass characteristics. Proc. V World Congress on Genetics Applied to Livestock Production (Guelph, Canada). 18:51-54.
Snowder, G. D., M. B. Solomon, S. K. Duckett, and N. E. Cockett. 1998. Changes in fat, bone and fat free lean tissue in callipyge and normal lambs across the growth curve. Proc. Amer. Soc. Anim. Sci. 76 (Abstr.):113.
Snyder, J. L., J. A. Clapper, A. J. Roberts, D. W. Sanson, D. L. Hamernik, and G. E. Moss. 1999. Insulin-like growth factor-I, insulin-like growth factor-binding proteins, and gonadotropins in the hypothalamic-pituitary axis and serum of nutrient-restricted ewes. Biol. Reprod. 61:219-224.
Vernon, R. G. 1976. Effect of dietary fats on ovine adipose tissue metabolism. Lipids 11:662-669.
Vernon, R. G. and E. Taylor. 1986. Acetyl-CoA carboxylase of sheep adipose tissue: problems of the assay and adaptation during fetal development. J. Anim. Sci. 63:1119-1125.
Whisnant, C. S., R. S. Kline, J. C. Branum, G. M. Zaunbrecher, M. Z. Khan, and S. P. Jackson. 1998. Hormonal profiles of callipyge and normal sheep. J. Anim. Sci. 76:1443-1447.